(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(5) *Residue on ignition.* Proceed as directed in §436.207(a) of this chapter.

(6) *Identity.* (i) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine, boil for 1 minute, and cool; a blue color develops; and

(ii) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10), mix well, and add, dropwise, 5 drops of a cupric sulfate solution (1:100) mixing after the addition of each drop; a reddish-violet color is produced.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

§448.930b Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant is polymyxin B sulfate and benzalkonium chloride, with one or more suitable and harmless suspending agents, in a suitable and harmless base. It contains, in each gram, 5,000 units of polymyxin B and 330 micrograms of benzalkonium chloride. Its content of polymyxin B is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 4.0 and not more than 5.5. The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1), except sterility, pyrogens, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, pH, loss on drying, residue on ignition, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch.

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20(e)(1) of this chapter, except dissolve the ointment as follows: Aseptically transfer a portion of 0.25 gram from each of 10 immediate containers of the drug to 400 milliliters of diluting fluid D in an Erlenmeyer flask. Repeat the procedure on another 10 immediate containers. Swirl the flasks to dissolve the ointment.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted sample.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

PART 449—ANTIFUNGAL ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.

AUTHORITY: Sec. 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357).

SOURCE: 39 FR 19134, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§§ 449.1—449.3 [Reserved]

§ 449.4 Amphotericin B.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH. 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 15 percent of amphotericin A.

(iii) [Reserved]

(iv) Its loss on drying is not more than 5.0 percent.

(v) It contains not more than 3.0 percent residue on ignition.

(vi) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5(b) of this chapter, each package shall bear on its label the statements ''Store below 10° C.'' and ''Protect from light and moisture''.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of test and assays on the batch for potency, amphotericin A content, loss on drying, residue on iginition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot; dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to

the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content*—(i) *Amphotericin A.* Dry approximately 20 milligrams of the nystatin working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 200-milliliter volumetric flask. Add exactly 40.0 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(ii) *Amphotericin B.* Dry approximately 50 milligrams of the amphotericin B working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly. The standard solution should be used for 1 day only.

(iii) *Sample.* Accurately weigh about 50 milligrams of the sample to be tested and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(iv) *Blank.* Pipette 10 milliliters of dimethylsulfoxide into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix thoroughly.

(v) *Procedure.* Use a suitable ultraviolet spectrophotometer and 1-centimeter silica cells. Adjust the instrument to zero with the blank solution. Measure the absorbances of the solutions of nystatin standard, amphotericin B standard, and the sample at 304 nanometers and at 282 nanometers. Calculate the absorptivity of each standard at both wavelengths:

$$\text{Percent amphotericin A} = \frac{[(B \times S_2) - (b \times S_1)] \times 625}{W_s \times [(B \times a) - (b \times A)]}$$

where:

A=Absorptivity of nystatin standard at 282 nanometers;
B=Absorptivity of amphotericin B standard at 282 nanometers;
a=Absorptivity of nystatin standard at 304 nanometers;
b=Absorptivity of amphotericin B standard at 304 nanometers;
S_1=Absorbance of sample at 282 nanometers;
S_2=Absorbance of sample at 304 nanometers;
W_s=Weight of sample in grams (on an anhydrous basis).

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Using the solutions prepared as described in paragraphs (b)(2) (ii), (iii), and (iv) of this section, record the absorption spectrum from 320 to 240 nanometers. Then dilute these solutions (1+9) with methyl alcohol and record the absorption spectrum from 400 to 320 nanometers. The sample exhibits absorption peaks at identical wavelengths with that of the amphotericin B standard. Depending on the amphotericin A content of the sample, a peak may occur at 304 nanometers.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 49 FR 2242, Jan. 19, 1984; 50 FR 19920, May 13, 1985]

§ 449.4a Amphotericin B for use in parenteral products.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow

to golden-orange powder. It is insoluble in water at pH 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 5 percent of amphotericin A.

(iii) [Reserved]

(iv) Its loss on drying is not more than 5.0 percent.

(v) It contains not more than 0.5 percent residue on ignition.

(vi) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by §432.5(b) of this chapter, each package shall bear on its label the statements ''Store below 10° C.'' and ''Protect from light and moisture''.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, amphotericin A content, loss on drying, residue on ignition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute with dimethylsulfoxide to give a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content.* Proceed as directed in §449.4(b)(2).

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(5) *Residue of ignition.* Proceed as directed in §436.207(a) of this chapter.

(6) *Identity.* Proceed as directed in §449.4(b)(7).

[39 FR 19134, May 30, 1974, as amended at 49 FR 2243, Jan. 19, 1984; 50 FR 19920, May 13, 1985]

§449.10 Candicidin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin is a brown to yellow powder. It is sparingly soluble in water; very slightly soluble in ethyl alcohol, butyl alcohol, and acetone. It is so purified and dried that:

(i) Its potency is not less than 1,000 micrograms of candicidin per milligram on an anhydrous basis.

(ii) Its loss on drying is not more than 4 percent.

(iii) Its pH is not less than 8.0 nor more than 10.0 in a 1 percent aqueous suspension.

(iv) Its ultraviolet absorption spectrum is characteristic of a conjugated heptaene and is qualitatively the same as that of the candicidin working standard.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve a portion of the sample in sufficient dimethylsulfoxide to yield an estimated concentration of 1,000 micrograms of candicidin activity per milliliter. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin activity per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using a 1 percent aqueous suspension.

(4) *Identity*—(i) *Preparation of aqueous alcohol solution.* Prepare an aqueous alcohol solution by mixing 53 volumes of ethyl alcohol and 47 volumes of water.

(ii) *Preparation of standard solution.* Grind a small portion of the candicidin working standard to a fine powder with a mortar and pestle. Accurately weigh an amount equivalent to 20,000 micrograms of candicidin activity and transfer it to a 100-milliliter volumetric flask. Add about 50 milliliters of the aqueous alcohol solution and shake to effect complete dissolution. Bring to volume with the aqueous alcohol solution and mix well. Transfer a 25-milliliter aliquot to a 100-milliliter volumetric flask and bring to volume with the aqueous alcohol solution. This solution contains 50 micrograms of candicidin activity per milliliter.

(iii) *Preparation of sample solution.* Proceed as directed in paragraph (b)(4)(ii) of this section.

(iv) *Procedure.* Using a suitable recording spectrophotometer, record the absorption spectra of the standard solution and the sample solution between the wavelengths of 330 and 410 nanometers with the aqueous alcohol solution as the reference solution. Compare the absorption spectra of the standard solution and the sample solution. They should exhibit absorption maxima and minima at the same wavelengths, which are approximately 342, 359, 378, and 397 nanometers for the maxima and 348, 366, and 390 nanometers for the minima.

[39 FR 19134, May 30, 1974, as amended at 44 FR 30333, May 25, 1979; 49 FR 2243, Jan. 19, 1984]

§ 449.20 Griseofulvin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin is a microsize, white to pale-cream compound with the following chemical name: 7-chloro-2′,4,6-trimethoxy-6′β-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione. It is so purified and dried that:

(i) Its griseofulvin content is not less than 900 micrograms and not more than 1,050 micrograms of griseofulvin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its melting point, after drying, is not less than 217° C. and not more than 224° C.

(v) Its specific rotation in dimethylformamide at 25° C. is not less than +348° and not more than +364°.

(vi) Its ultraviolet absorption spectrum in methyl alcohol compares qualitatively with that of the griseofulvin reference standard.

(vii) Its residue on ignition is not more than 0.2 percent.

(viii) Its heavy metals content is not more than 25 parts per million.

(ix) Its specific surface area is not less than 1.3 and not more than 1.7 square meters per gram.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 1 gram.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter.

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *Melting point.* Proceed as directed in § 436.209 of this chapter.

(5) *Specific rotation.* Accurately weigh approximately 250 milligrams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of dimethylformamide. Bring to volume with dimethylformamide, stopper, and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(6) *Identity.* Dissolve an accurately weighed portion of the sample and of the griseofulvin working standard and dissolve each in sufficient methyl alcohol to obtain a concentration of 10

micrograms of griseofulvin per milliliter and mix well. (The standard solution can be kept under refrigeration and used for up to 1 month.) Record the ultraviolet absorption spectrum of solutions of the sample and standard from 240 to 320 nanometers. The spectral curves shall be similar, and each shall have a maximum at 292± 2 nanometers and a minimum at 269± 2 nanometers.

(7) *Residue on ignition.* Proceed as directed in § 436.207 of this chapter.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(9) *Specific surface area*—(i) *Procedure.* Determine the apparent particle size in microns by the air-permeation method, using a suitable subsieve sizer. Weigh 1.819 grams ±0.001 gram of the sample and transfer to the compression tube of the apparatus. Compact the sample with moderate pressure so that it has a uniform porosity. Pass compressed dry air through the sample and measure the air pressure with a water manometer. Observe the porosity and calculate the apparent particle size from the instrument equation or read it from a chart that has been calculated in accordance with the equation. Repeat the readings at successively higher degrees of compaction until the apparent particle size reaches a minimum. Calculate the observed specific surface area (SSA) in square meters per gram of sample, as follows:

$$\text{Observed SSA} = \frac{6}{\text{Minimum apparent particle size in microns} \times 1.455 \times F}$$

where F is a factor used to correct the apparent particle size to the true particle size:

Porosity reading	F
0.80	1.3771
0.76	1.4142
0.72	1.4573
0.68	1.5082
0.64	1.5690
0.60	1.6432
0.56	1.7353
0.52	1.8528
0.48	2.0076
0.44	2.2203
0.40	2.5298

(ii) Standard. Determine the observed specific surface area of the griseofulvin specific surface area standard by the method prescribed in paragraph (b)(9)(i) of this section, using the same instrument and the same air pressure setting.

(iii) *Calculations.* Calculate the corrected specific surface area of the sample as follows:

$$\text{SSA of sample} = \frac{\text{Observed SSA of sample} \times \text{assigned SSA of standard}}{\text{Observed SSA of standard}}$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 44 FR 20660, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.40 Natamycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Natamycin is 22-[(3 - amino - 3,6 - dideoxy -β-D-mannopyranosyl) - oxy] - 1,3,26-trihydroxy - 12-methyl-10-oxo-6,11,28-trioxatricyclo [22.3.1.$0^{5,7}$] octacosa-8,14,16,18,20 - pentaene-25-carboxylic acid. It is an off-white to cream colored powder which may contain up to 3 moles of water. It is practically insoluble in water, slightly soluble in methanol, and soluble in glacial acetic acid and dimethylformamide. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of natamycin per milligram on an anhydrous basis.

(ii) Its moisture content is not less than 6.0 percent and not more than 9.0 percent.

(iii) Its pH in a 1 percent aqueous suspension is not less than 5.0 and not more than 7.5.

(iv) It passes the identity test.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay.* Dilute solutions of natamycin are very sensitive to light and should be kept in the dark as much as possible or substantial decomposition will take place.

(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in dimethylsulfoxide and further dilute with sufficient dimethylsulfoxide to give a concentration of 100 micrograms of natamycin per milliliter (estimated). Further dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 5.0 micrograms of natamycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using a 1.0 percent aqueous suspension.

(4) *Identity.* Accurately weigh approximately 50 milligrams of the sample into a 200-milliliter volumetric flask. Add approximately 5.0 milliliters of distilled water, and completely moisten the sample. Then add approximately 100 milliliters of an acid-alcohol solvent (0.1 percent glacial acetic acid in methyl alcohol) and stir or shake mechanically in the dark until solution is complete. Dilute to volume with the acid-alcohol solvent. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with the acid-alcohol solvent. Using a suitable spectrophotometer with 1-centimeter cells and the acid-alcohol solvent as a blank, record the ultraviolet absorption spectrum from 215 to 330 nanometers. The spectrum compares qualitatively to that of the natamycin working standard similarly treated.

(5) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[43 FR 55384, Nov. 28, 1978, as amended at 46 FR 16684, Mar. 13, 1981]

§449.50 Nystatin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin is the yellow to light-tan compound of a kind of nystatin or a mixture of two or more such compounds. It is very slightly soluble in water, moderately soluble in methyl alcohol, butyl alcohol, or propyl alcohol. It is so purified and dried that:

(i) Its potency is not less than 4,400 units of nystatin per milligram; except, if it is packaged for extemporaneous preparation of oral suspensions, its potency is not less than 5,000 units of nystatin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5.0 percent.

(iv) Its pH in a 3 percent aqueous suspension is not less than 6.5 and not more than 8.0.

(v) It passes the identity test.

(vi) If it is packaged for extemporaneous preparation of oral suspensions, it passes the suspendibility test.

(vii) If it is packaged for extemporaneous preparation of oral suspensions, it is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity. In addition, if it is packaged for extemporaneous preparation of oral suspensions, results of tests and assays on the batch for suspendibility and crystallinity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylformamide to give a nystatin concentration of 400 units per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 3 percent aqueous suspension of the drug.

(5) *Identity.* Weigh approximately 100 milligrams of the sample into a 200-milliliter, glass-stoppered, volumetric flask. Add 50 milliliters of absolute methyl alcohol and 10 milliliters of glacial acetic acid. When the sample has dissolved, dilute to volume with methyl alcohol. Transfer 2 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with methyl alcohol. Use the same dilution of acetic acid in methyl alcohol as the blank. Immediately determine the absorption peaks at 230, 291, 305, and 319 nanometers, and the shoulders at 279±2 nanometers, using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100 percent transmission with the blank. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 220 nanometers to 350 nanometers. If a nonrecording spectrophotometer is used, the exact positions of the peaks and shoulder should be determined for the particular instrument used. The ratio of the two absorbances

$$(A_{230}/A_{279})$$

should be not less than 0.90 and not more than 1.25.

(6) *Suspendibility test.* Transfer 200 milligrams of the sample into a 250-milliliter beaker containing 200 milliliters of water. Swirl the suspension gently with a stirring rod. Allow the beaker to remain still for 2 minutes and observe the bottom. It passes the test if the powder remains in suspension. If a significant amount of sediment is observed, withdraw an accurately measured aliquot of the undisturbed suspension and assay as directed in § 449.150c(b)(1) of this chapter. It passes the test if the suspension contains not less than 90 percent of the number of units of nystatin that it is represented to contain.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 39 FR 43833, Dec. 19, 1974; 49 FR 5098, Feb. 10, 1984; 50 FR 19920, May 13, 1985]

Subpart B—Oral Dosage Forms

§ 449.104 Amphotericin B oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B oral suspension is a mixture of amphotericin B with one or more suitable and harmless preservatives, colorings, sweetening ingredients, flavorings, buffer substances, lubricants, suspending agents, and sequestrants in an aqueous vehicle. Each milliliter contains 100 milligrams of amphotericin B. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B that it is represented to contain. Its pH is not less than 4.5 and not more than 6.0. The amphotericin B conforms to the standards prescribed by § 449.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, loss on drying, pH, residue on ignition, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The amphotericin B used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion into a high-speed glass blender with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot of the stock solution with dimethylsulfoxide to give a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Further dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *pH*. Proceed as directed in §436.202 of this chapter, using the undiluted suspension.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§449.120 Griseofulvin oral dosage forms.

§449.120a Griseofulvin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Griseofulvin tablets are tablets composed of griseofulvin, with or without one or more suitable fillers, colorings, lubricants, and binders. Each tablet contains 125, 250, or 500 milligrams of griseofulvin. The griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 5.0 percent. The tablets shall disintegrate within 1 hour. The griseofulvin used conforms to the standards prescribed by §449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(*b*) The batch for griseofulvin content, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch for griseofulvin content, loss on drying, and disintegration time.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in §436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Accurately weigh 20 tablets and determine the average tablet weight. Grind the tablets to a fine powder in a mortar and transfer an accurately weighed sample to a volumetric flask of such size that for each 50 milliliters of volume there are 40 milligrams of griseofulvin (estimated). Add chloroform to about one-fourth volume of the flask. Swirl the flask and apply gentle heat to aid in dissolution of the griseofulvin. Allow the mixture to cool and then dilute to volume with chloroform and mix. Allow to settle and transfer 2.0 milliliters of the supernate to a conical centrifuge tube and evaporate to dryness under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per tablet as follows:

$$\text{Milligrams of griseofulvin per tablet} = \frac{R_u \times W_s \times f \times W_a \times V_u}{R_s \times W_u \times 1{,}000 \times 50}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

f=Potency of the griseofulvin working standard in micrograms per milligram;

W_a=Average tablet weight in milligrams;

W_s=Weight of the griseofulvin working standard in milligrams;

W_u=Weight of the ground tablet powder sample in milligrams;

V_u=Volume of the dissolved ground tablet powder sample in milliliters.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[39 FR 19134, May 30, 1974, as amended at 44 FR 20660, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.120b Griseofulvin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin capsules are gelatin capsules containing griseofulvin with a suitable filler and binder, with or without a suitable lubricant. Each capsule contains 125 or 250 milligrams of griseofulvin. The griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 1.0 percent. The griseofulvin used conforms to the standards prescribed by § 449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(*b*) The batch for griseofulvin content and loss on drying.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Empty the contents of 20 capsules into a tared weighing bottle. Weigh the powder and calculate the average capsule weight. Mix the powder and transfer an accurately weighed sample to a volumetric flask of such size that for each 50 milliliters of volume there are 40 milligrams of griseofulvin (estimated). Add chloroform to about one-fourth volume of the flask. Swirl the flask and apply gentle heat to aid in dissolution of the griseofulvin. Allow the mixture to cool and then dilute to volume with chloroform and mix. Allow to settle and transfer 2.0 milliliters of the supernate to a conical centrifuge tube and evaporate to dryness under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per capsule as follows:

$$\text{Milligrams of griseofulvin per capsule} = \frac{R_u \times W_s \times f \times W_a \times V_u}{R_s \times W_u \times 1{,}000 \times 50}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

W_s=Weight of the griseofulvin working standard in milligrams;

f=Potency of the griseofulvin working standard in micrograms per milligram;

W_a=Average capsule fill weight in milligrams;

W_u=Weight of the ground tablet powder sample in milligrams;

V_u=Volume of the dissolved capsule powder sample in milliliters.

(2) *Loss of drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 43 FR 9800, Mar. 10, 1978; 44 FR 20661, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.120c Griseofulvin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin oral suspension is griseofulvin oral suspension with one or more suitable flavorings, colorings, wetting agents, preservatives, and diluents in an aqueous vehicle. Each milliliter contains 25 milligrams of griseofulvin. Its griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of

the number of milligrams of griseofulvin that it is represented to contain. Its pH is not less than 6.5 and not more than 7.5. The griseofulvin used conforms to the standards prescribed by §449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(*b*) The batch for griseofulvin content and pH.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in §436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Transfer an accurately measured portion of the oral suspension equivalent to 100 milligrams of griseofulvin into a 50-milliliter round-bottomed glass-stoppered centrifuge tube. Add 5 milliliters of water and 20 milliliters of a solvent mixture of ethyl acetate and chloroform (85:15). Shake the tube for 1 minute and centrifuge it briefly to separate the layers. Transfer most of the upper layer to a 100-milliliter volumetric flask being careful not to remove any of the lower aqueous layer. Repeat the extraction step with two additional 20-milliliter portions of the solvent mixture combining the extracts in the volumetric flask with the first 20-milliliter extract. Dilute to volume with the solvent mixture and mix. Place 2.0 milliliters of this solution in a conical centrifuge tube and evaporate the contents to dryness on a steam bath under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per milliliter as follows:

$$\text{Milligrams of griseofulvin per milliliter} = \frac{R_u \times W_s \times f \times 2}{R_s \times 1{,}000 \times V_o}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

W_s=Weight of the griseofulvin working standard in milligrams;

f=Potency of the griseofulvin working standard in micrograms per milligram;

V_o=Volume of oral suspension taken in milliliters.

(2) *pH.* Proceed as directed in §436.202 of this subchapter, using the undiluted suspension.

[39 FR 19134, May 30, 1974, as amended at 44 FR 20662, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§449.120d Griseofulvin (ultramicrosize) tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin (ultramicrosize) tablets are composed of ultramicrosize crystals of griseofulvin which may or may not be dispersed in polyethylene glycol 6,000. Each tablet contains 125, 165, 250, or 330 milligrams of griseofulvin. The griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 5.0 percent. It passes the solubility characteristic test. If it is dispersed in polyethylene glycol 6,000, the griseofulvin used conforms to the standards prescribed by §449.20(a)(1). If it is not dispersed in polyethylene glycol 6,000, the griseofulvin used conforms to the standards prescribed by §449.20(a)(1), except specific surface area.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for potency, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area (if it is dispersed in polyethylene glycol 6,000), and crystallinity.

(*b*) The batch for griseofulvin content, loss on drying, and solubility characteristic.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in §436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Accurately weigh 20 tablets and determine the average tablet weight. Grind the tablets to a fine powder in a mortar and transfer an accurately weighed sample to a volumetric flask of such size that for each 50 milliliters of volume there are 40 milligrams of griseofulvin (estimated). Add chloroform to about one-fourth volume of the flask. Swirl the flask and apply gentle heat to aid in dissolution of the griseofulvin. Allow the mixture to cool and then dilute to volume with chloroform. Mix and allow to settle. Using gentle vacuum, remove and discard the waxy substance that forms on the top of the chloroform. Transfer 2.0 milliliters of the chloroform solution to a conical centrifuge tube and evaporate to dryness under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per tablet as follows:

$$\text{Milligrams of griseofulvin per tablet} = \frac{R_u \times W_s \times f \times W_a \times V_u}{R_s \times W_u \times 1{,}000 \times 50}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

W_s=Weight of the griseofulvin working standard in milligrams;

f=Potency of the griseofulvin working standard in micrograms per milligram;

W_a=Average tablet weight in milligrams;

W_u=Weight of the ground tablet powder sample in milligrams;

V_u=Volume of the dissolved ground tablet powder sample in milliliters.

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(3) *Solubility characteristic test.* Proceed as directed in §436.317 of this chapter.

[40 FR 41523, Sept. 8, 1975, as amended at 43 FR 22676, May 26, 1978; 46 FR 7275, Jan. 23, 1981; 46 FR 21361, Apr. 10, 1981; 46 FR 46313, Sept. 18, 1981; 47 FR 34132, Aug. 6, 1982; 50 FR 19920, May 13, 1985]

§ 449.150 Nystatin oral dosage forms.

§ 449.150a Nystatin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin tablets are tablets composed of nystatin and suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 500,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The loss on drying is not more than 8 percent. The tablets shall disintegrate within 2 hours. The nystatin used conforms to the standards prescribed by §449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985; 50 FR 52772, Dec. 26, 1985]

§449.150b Nystatin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin oral suspension is a suspension containing nystatin and one or more suitable preservatives, suspending agents, surfactants, flavorings, and colorings in purified water. Each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its pH is not less than 4.5 and not more than 6.0; except, if the product contains glycerin, its pH is not less than 6.0 and not more than 7.5. The nystatin used conforms to the standards prescribed by §449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative aliquot of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Remove and dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted suspension.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§449.150c Nystatin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin for oral suspension is a dry powder consisting of nystatin, and suitable and harmless suspending substances, preservatives, diluents, colorings, and flavorings. When the suspension is prepared as directed in its labeling, each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of aliquot of the stock solution and further the number of units of nystatin that it is represented to contain. The pH of the reconstituted drug is not less than 4.9 and not more than 5.5. Its moisture content is not more than 7.0 percent. The nystatin used conforms to the standards prescribed by §449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of

§ 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, moisture and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Blend an appropriate aliquot in a high-speed glass blender for 3 to 5 minutes, using sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture.* Using the dry powder, proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension after reconstituting as directed in the labeling.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 449.150d Nystatin pastilles.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin pastilles are composed of nystatin with suitable diluents, binders, buffers, colorings, and flavorings. Each pastille contains nystatin equivalent to 200,000 units of nystatin. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units of nystatin that it is represented to contain. The pH in an aqueous solution is not less than 5.0 and not more than 7.5. It disintegrates within 90 minutes. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, pH, and disintegration time.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 pastilles.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of pastilles into a high-speed glass blender jar containing 100 milliliters of sterile distilled water. Blend for 18 to 20 minutes. Add 400 milliliters of dimethylformamide and continue blending for an additional 10 minutes. Remove an aliquot and add sufficient 80 percent dimethylformamide so that upon final dilution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter, the concentration of dimethylformamide will be 4 percent.

(2) *pH.* Dissolve 1 pastille in 100 milliliters of distilled water at 37 °C, cool, and proceed as directed in § 436.202 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the method described in paragraph (e)(4) of that section.

[52 FR 4617, Feb. 13, 1987; 52 FR 7741, Mar. 12, 1987, as amended at 55 FR 11584, Mar. 29, 1990]

Subpart C—Injectable Dosage Forms

§ 449.204 Amphotericin B for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B for injection is a dry mixture containing in

each immediate container 50 milligrams of amphotericin B, 41 milligrams of sodium desoxycholate, and suitable buffering substances. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of amphotericin B that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 8.0 percent. Its pH in an aqueous solution containing 10 milligrams of amphotericin B per milliliter is not less than 7.2 and not more than 8.0. The amphotericin B used conforms to the standards prescribed by § 449.4a(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, each package shall bear on its label and labeling the following statement: ''For intravenous infusion in hospitals only''.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, loss on drying, pH, residue on ignition, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then using a suitable syringe and hypodermic needle, remove all of the withdrawable contents if the container is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot of this solution and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(e) of this chapter, using a solution containing 2 milligrams of amphotericin B per milliliter, except in lieu of paragraph (a)(3), if no rabbit shows an individual rise in temperature of 1.1° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 3° C., the sample meets the requirements for absence of pyrogen. If one or two rabbits show a temperature rise of 1.1° C. or more, or if the sum of temperature rises exceeds 3° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show a temperature rise of 1.1° C. or more, and if the sum of the temperature rises does not exceed 8° C. the sample meets the requirements for absence of pyrogens.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter using an aqueous solution containing 10 milligrams of amphotericin B per milliliter.

[39 FR 19134, May 30, 1974, as amended at 45 FR 16472, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

Subpart D—Ophthalmic Dosage Forms

§ 449.340 Natamycin ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Natamycin ophthalmic suspension contains natamycin with one

or more suitable and harmless preservatives in a suitable and harmless aqueous vehicle. Each milliliter contains 50 milligrams of natamycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of natamycin that it is represented to contain. It is sterile. Its pH is not less than 5.0 and not more than 7.5. The natamycin used conforms to the standards prescribed by § 449.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The natamycin used in making the batch for potency, moisture, pH, identity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The natamycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Dilute solutions of natamycin are very sensitive to light and should be kept in the dark as much as possible or substantial decomposition will take place.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with dimethylsulfoxide to a concentration of 100 micrograms of natamycin per milliliter (estimated). Further dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 5.0 micrograms of natamycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of sample in lieu of 1.0 milliliter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

[43 FR 55384, Nov. 28, 1978, as amended at 48 FR 51293, Nov. 8, 1983]

Subpart E—[Reserved]

Subpart F—Dermatologic Dosage Forms

§ 449.504 Amphotericin B dermatologic dosage forms.

§ 449.504a Amphotericin B ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B ointment is composed of amphotericin B in a suitable and harmless ointment base. It may contain suitable and harmless coloring agents and protectants. It contains 30 milligrams of amphotericin B in each gram. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B that it is represented to contain. Its moisture content is not more than 1.0 percent. The amphotericin B used conforms to the standards prescribed by § 449.4(a)(1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately

weighed representative portion of the sample (usually 1 gram) into an appropriate-sized Erlenmeyer flask with 10 milliliters of ethyl ether. Allow to dissolve for 1 hour with the intermittent manual shaking. Add a measured amount of dimethylsulfoxide to the flask and place on a shaker for 10 minutes. Further dilute with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

§449.504b Amphotericin B cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B cream is composed of amphotericin B, with or without one or more suitable and harmless emollients, perfumes, dispersants, and preservatives, in a suitable and harmless cream base. It contains 30 milligrams of amphotericin B in each gram. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B per gram that it is represented to contain. The amphotericin B used conforms to the standards prescribed by §449.4(a)(1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay; potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: With the aid of a high-speed glass blender, dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

§449.504c Amphotericin B lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B lotion is composed of amphotericin B in a suitable and harmless lotion vehicle. It contains suitable and harmless emollients, emulsifiers, coloring agents, diluents, preservatives, and perfumes. It contains 30 milligrams of amphotericin B per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B per milliliter that it is represented to contain. Its pH is not less than 5.0 and not more than 7.0. The amphotericin B used conforms to the standards prescribed by §449.4(a)(1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an aliquot in

sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute the stock solution with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted lotion.

§ 449.550 Nystatin dermatologic dosage forms.

§ 449.550a Nystatin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin ointment is composed of nystatin and a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give a concentration of 400 units of nystatin (estimated) per milliliter, blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.550b Nystatin-iodochlorhydroxyquin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-iodochlorhydroxyquin ointment is composed of nystatin and iodochlorhydroxyquin in a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin and 10 milligrams of iodochlorhydroxyquin. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its iodochlorhydroxyquin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of iodochlorhydroxyquin that it is represented to contain. It passes the identity test for iodochlorhydroxyquin. Its moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1). The iodochlorhydroxyquin used conforms to the standards prescribed by U.S.P. XVIII.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The iodochlorhydroxyquin used in making the batch for all U.S.P. XVIII specifications.

(*c*) The batch for nystatin content, iodochlorhydroxyquin content, iodochlorhydroxyquin identity, and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute an aliquot of the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Iodochlorhydroxyquin content*—(i) *Reagents.* (*a*) Ferric chloride reagent. Dissolve 1.0 gram of ferric chloride ($FeCl_3 \cdot 6H_2O$) in a mixture of 1.0 milliliter of concentrated hydrochloric acid and sufficient distilled water to make 1 liter.

(*b*) Acetone, reagent grade.

(*c*) 2-Methoxyethanol, reagent grade.

(ii) *Preparation of standard solution.* Dissolve an accurately weighed portion of iodochlorhydroxyquin U.S.P. reference standard in sufficient 2-methoxyethanol to make a solution containing 1.0 milligram of iodochlorhydroxyquin per milliliter. Transfer 5.0 milliliters of this standard solution to a 50-milliliter volumetric flask.

(iii) *Preparation of sample solution.* Accurately weigh a portion of the sample equivalent to 50 milligrams of iodochlorhydroxyquin into a 125-milliliter Erlenmeyer flask. Add 50 milliliters of acetone, warm on a steam bath, and shake gently. Cool to room temperature and filter contents through a pledget of glass wool into a 100-milliliter volumetric flask. Wash the Erlenmeyer flask with two 20-milliliter portions of acetone and filter the washings into the volumetric flask. Dilute to volume with acetone and mix thoroughly. Transfer a 10-milliliter aliquot of the acetone solution to a 50-milliliter volumetric flask and evaporate on a steam bath. To the residue, add 20 milliliters of 2-methoxyethanol and swirl to dissolve the iodochlorhydroxyquin.

(iv) *Procedure.* To each flask containing standard solution and sample solution, respectively, add 2.0 milliliters of ferric chloride reagent and dilute to volume with 2-methoxyethanol. Mix thoroughly. Using a suitable spectrophotometer equipped with 1.0-centimeter cells and a blank prepared by diluting 2.0 milliliters of ferric chloride reagent to 50 milliliters with 2-methoxyethanol, determine the absorbance of the sample and standard solutions at 650 nanometers. Set the instrument to 100-percent transmission with the blank.

(v) *Calculation.*

Milligrams of iodochlorhydroxyquin per gram of sample=(Absorbance of sample × 50)/(Absorbance of standard × Weight of sample in grams)

(3) *Iodochlorhydroxyquin identity.* Proceed as directed in § 436.400 of this chapter, preparing the sample solution as follows: Accurately weigh a portion of the sample equivalent to 50 milligrams of iodochlorhydroxyquin into a 125-milliliter Erlenmeyer flask. Add 50 milliliters of acetone, warm on a steam bath, and shake gently. Cool to room temperature and filter contents through a pledget of glass wool into a 100-milliliter volumetric flask. Wash the Erlenmeyer flask with two 20-milliliter portions of acetone and filter the washings into the volumetric flask. Dilute to volume with acetone and mix thoroughly.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 449.550c Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide ointment; nystatin-neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is nystatin, neomycin sulfate, gramicidin, and either triamcinolone acetonide or fludrocortisone acetate in a suitable ointment base. Each gram contains

100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and either 1.0 milligram of triamcinolone acetonide or 1.0 milligram of fludrocortisone acetate. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. Its moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by §449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) of this chapter. The gramicidin used conforms to the standards prescribed by §448.25(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes with sufficient dimethylformamide to give a concentration of 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogenous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Accurately weigh and dissolve a representative portion of the sample in approximately 50 milliliters of petroleum ether in a separatory funnel. Extract with 20 milliliters of 80 percent alcohol prepared from alcohol U.S.P. XX. Repeat the extraction three times. Combine the extractives in a suitable volumetric flask, bring to volume with alcohol U.S.P. XX, and mix well. Further dilute with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§ 449.550d Nystatin cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin cream is composed of nystatin and suitable and harmless emulsifiers, perfumes, buffers, preservatives, and a protectant in a suitable and harmless cream base. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give an estimated concentration of 400 units of nystatin per milliliter, blend an accurately weighed representative portion in a high-speed blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

§ 449.550e Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream is composed of nystatin, neomycin sulfate, gramicidin, triamcinolone acetonide, and suitable and harmless emulsifiers, solvents, perfumes, buffers, preservatives, and a protectant in a suitable cream base. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1 milligram of triamcinolone acetonide. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 444.42(a)(1) of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for nystatin content, neomycin content, and gramicidin content.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give a concentration of 400 units of nystatin (estimated) per milliliter, blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the cream in a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and either until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to ensure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Accurately weigh a representative portion of the sample and dissolve in approximately 50 milliliters of petroleum ether in a separatory funnel. Extract with 20 milliliters of 80 percent alcohol prepared from alcohol U.S.P. XX. Repeat the extraction three times. Combine the extractives in a suitable volumetric flask, bring to volume with alcohol U.S.P. XX, and mix well. Further dilute with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

[39 FR 19134, May 30, 1974, as amended at 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§ 449.550f Nystatin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin topical powder is a dry powder composed of nystatin and talc. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its loss on drying is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by §449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative sample for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute with sufficient

dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 449.550g Nystatin-neomycin sulfate-gramicidin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin topical powder is a dry powder composed of nystatin, neomycin sulfate, gramicidin, and talc. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin and 0.25 milligram of gramicidin. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. Its loss on drying is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v) and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for nystatin content, neomycin content, gramicidin content, and loss on drying.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend the entire contents of an accurately weighed representative portion of the sample for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative sample for 3 to 5 minutes in sufficient 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to give a convenient concentration. Further dilute an aliquot with solution 3 to the reference concentration of 1 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed representative sample in alcohol U.S.P. XX and filter. Collect the filtrate and dilute a portion with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§ 449.550h Nystatin lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin lotion is composed of nystatin with one or more suitable and harmless suspending agents, emulsifiers, surfactants, and preservatives in a suitable and harmless vehicle. Each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its pH is not less than 5.5 and not more than 7.5. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[40 FR 3766, Jan. 24, 1975, as amended at 50 FR 19920, May 13, 1985]

Subpart G—Vaginal Dosage Forms

§ 449.610 Candicidin vaginal dosage forms.

§ 449.610a Candicidin vaginal ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal ointment is composed of candicidin and a suitable ointment base. It contains 0.6 milligram of candicidin per gram. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of candicidin that it is represented to contain. Its moisture content is not more than 0.1 percent. The candicidin used conforms to the requirements of § 449.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The candicidin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of *n*- hexane (containing 0.1 percent butylated hydroxyanisole). Shake the sample and *n*- hexane until homogeneous. Add 15 milliliters of

dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole) and shake well. Allow the layers to separate. Remove the bottom layer and repeat the extraction procedure with a second 15-milliliter portion of dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole). Combine the extractives in a suitable volumetric flask and fill to volume with sterile distilled water. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 40 FR 15089, Apr. 4, 1975]

§449.610b Candicidin vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal tablets are tablets composed of candicidin with suitable binders, diluents, and lubricants. Each tablet contains 3 milligrams of candicidin. Its potency is satisfactory if it is not less than 90 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain, except that for the issuance of a certificate for each batch, the candicidin content must be not less than 115 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain. The tablets shall disintegrate within 30 minutes. The loss on drying is not more than 1 percent. The candicidin used in making the batch conforms to the standards of §449.10(a)(1).

(2) *Labeling.* The drug shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) *Samples required.* (*a*) The candicidin used in making the batch: 10 packages, each consisting of approximately 300 milligrams.

(*b*) The batch: A minimum of 56 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Weigh a pool of five tablets and grind in a mortar to a very fine powder. Suspend an accurately weighed aliquot (of approximately 2 grams) in 10 milliliters of dimethylsulfoxide. Centrifuge for 5 minutes at 2,000 revolutions per minute. Carefully decant the supernatant solution into a sterile 250-milliliter volumetric flask. Wash the residue three times with 5-milliliter portions of dimethylsulfoxide, centrifuging each time. Add the washes to the 250-milliliter volumetric flask and fill to volume with sterile distilled water. Using sterile distilled water, further dilute to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the method described in paragraph (e)(1) of that section, except use distilled water as the immersion fluid.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

§449.610c Candicidin vaginal capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal capsules are gelatin capsules containing 3 milligrams of candicidin in a suitable and harmless ointment. The candicidin content is satisfactory if it is not less than 90 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain. The moisture content is not more than 0.1 percent. The candicidin used conforms to the requirements of §449.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The candicidin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 20 capsules.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Remove the tips from two capsules and express the ointment from each capsule into a separatory funnel containing approximately 50 milliliters of *n*- hexane (containing 0.1 percent butylated hydroxyanisole). Wash out the capsules at least two times with 2- to 3-milliliter portions of warm (approximately 50° C) *n*- hexane (containing 0.1 percent butylated hydroxyanisole). Add the washes to the separatory funnel. Shake the sample and *n*- hexane until homogeneous. Add 15 milliliters of dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole) and shake well. Allow the layers to separate. Remove the bottom layer and repeat the extraction procedure with a second 15-milliliter portion of dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole). Combine the extractives in a suitable volumetric flask and fill to volume with sterile distilled water. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 40 FR 15089, Apr. 4, 1975]

§ 449.650 Nystatin vaginal dosage forms.

§ 449.650a Nystatin vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Nystatin vaginal tablets are tablets composed of nystatin and suitable and harmless diluents, binders, and lubricants. Each tablet contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. The loss on drying is not more than 5 percent. The disintegration time is not more than 1 hour. The nystatin used conforms to the standards prescribed therefor by § 449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for nystatin content, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 immediate containers of approximately 300 milligrams each.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section, except use distilled water in lieu of gastric fluid.

[39 FR 19134, May 30, 1974. Redesignated at 43 FR 43458, Sept. 26, 1978]

§ 449.650b Nystatin vaginal suppositories.

(a) *Requirements for certification.*—(1) *Standards of identity, strength, quality,*

and purity. Nystatin vaginal suppositories contain in each suppository 100,000 units of nystatin in a suitable and harmless water soluble base. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its moisture content is not more than 1.5 percent. The nystatin used conforms to the standards prescribed by §449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 suppositories.

(b) *Tests and methods of assay.*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of suppositories into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot with sufficient dimethylformamide to obtain a concentration of 400 units of nystatin per milliliter (estimated). Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[43 FR 43458, Sept. 26, 1978, as amended at 50 FR 19920, May 13, 1985]

PART 450—ANTITUMOR ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Subpart B—[Reserved]

Subpart C—Injectable Dosage Forms

AUTHORITY: Sec. 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357).

Subpart A—Bulk Drugs

§450.10a Sterile bleomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile bleomycin sulfate is the amorphous sulfate salt of bleomycin. Bleomycin has been separated into several similar glyco-peptide molecules. It is a cream-colored powder that is so purified and dried that:

(i) Its potency is not less than 1.5 units and not more than 2.0 units of bleomycin per milligram. If it is packaged for dispensing, the content of the ampoule or vial is not less than 90 percent and not more than 120 percent of the number of units of bleomycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]